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Forest Pest Management Report

R-3 83-10

BIOLOGICAL EVALUATION
Effects of Soil Fumigation at
Albuquerque Tree Nursery on Soil-Borne
Plant Pathogens and Seedlings

Cibola National Forest
New Mexico

April 1983

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USDA Forest Service, Southwestern Region
State and Private Forestry
Forest Pest Management
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INTRODUCTION

The Albuquerque Tree Nursery (ATN) annually fumigates seedbeds for the purpose of suppressing potential soil-borne plant pathogens, weeds, and nematodes. Since 1979, the Forest Pest Management Staff has evaluated the effectiveness of the annual fumigation in reducing populations of various fungi, nematodes, and weed seeds. The earlier (1979, 1980) evaluations indicated that the results of fumigation were variable and inconclusive. During these years, ATN personnel were testing various combinations of treatment methods and times on blocks having different soils and cultural histories. In addition, plots used to collect data for the evaluation were located at row ends, where they were subject to damage by equipment, herbicides, and foraging animals. In 1981, block 1 was treated in the spring, using the alternate strip method, and our plots were located inside the block. Levels of Fusarium and Pythium were very low after fumigation, and seedling survival was significantly higher than in unfumigated plots at the end of the growing season, indicating that the treatment objectives were accomplished.

The same combination of fumigation methods and timing was used in 1982 as in 1981. Because the previous evaluations had found no pathogenic nematodes, charcoal root rot, or Phytophthora, the 1982 evaluation was limited to determining the effects of fumigation on the fungal pathogens Fusarium and Pythium, and on seedling survival, over the growing season.

METHODS

The effects of fumigation on Fusarium and Pythium were determined by collecting soil samples shortly before and 2 weeks after fumigation was done in early May, and at the end of the growing season in September. We walked the length of each section, inserting a 1-inch diameter soil core sampler 4 to 6 inches into the soil about every 20 feet. The cores extracted in each section were combined and mixed, and a sample weighing about 1 kilogram was taken to the laboratory and treated using standard techniques and media selective for Fusarium and Pythium (appendix I).

The effects of fumigation on seedling survival were determined by establishing pairs of fumigated and unfumigated plots. Six sections in each of the two blocks were selected at random to contain plot pairs (figure 1). The fumigation crew was instructed to establish the unfumigated plots, each 24 by 44 feet, oriented east-west, in the selected sections between 200 and 250 feet from the north ends of the blocks. The actual distance was determined by the mechanics of fumigation, which is done in strips about 8 feet wide. The north sides of the unfumigated plots were 210 feet from the ends of sections 3, 4, 5, 7, 10, and 12 in block 7, and 240 feet from the ends of sections 1, 2, 3, 9, 11, and 15 in block 8. A wooden stake was placed at the corners of each unfumigated plot; later, orange flagging was tied around the

irrigation pipes marking the east and west ends of the plots. Areas of the same size and shape, located adjacent to, and on the north sides of, the unfumigated plots, served as fumigated plots.

Soil samples were taken, and estimates of seedling densities were made monthly between June and October, at random locations within strips 8 by 34 feet, centered in each plot. Soil samples were obtained and treated as outlined above; estimates of seedling density were made by placing a 1- by 4-foot frame across each bed in the plot and counting the live seedlings contained therein.

RESULTS AND DISCUSSION

Levels of Fusarium were relatively high prior to fumigation, averaging 2,141 propagules per gram (ppg) (table 1). The levels found after treatment, 98 ppg, were significantly lower at the 0.01-percent level of probability. The density of propagules had increased by the end of the growing season to approximately 353 ppg, but the increase is not statistically significant. A population of less than 500 ppg is considered too low to cause damage to seedlings.[1]

On the other hand, Pythium was found to be at a very low level before fumigation (averaging 2.15 ppg), was not significantly reduced by treatment, and was present at a low level at the end of the growing season (table 2). This pattern is consistent with results reported in previous years. The apparent lack of effect of fumigation on Pythium is possibly due to the sampling methods used, which are not designed to sample very small populations. Because of the low level of Pythium at ATN, we do not feel that the fungus constitutes a threat to seedling survival at this time, except possibly in areas having a high clay content where drainage is a problem.

In the fumigated plots used to determine seedling survival, levels of Fusarium and Pythium were similar to the average levels for the blocks as a whole. Fusarium levels were low (33.3 ppg) immediately after fumigation, but gradually and steadily increased to an average high of 300 ppg in August, then declined by about half in September (table 3 and figure 2). Density of Pythium varied greatly between samples and months, but was always low (table 4 and figure 3). Because we feel that Pythium is not a threat to seedlings at this time, further discussion will be confined to Fusarium.

In unfumigated soils, Fusarium started out in May at high overall levels of almost 2,000 ppg, increased slightly in June and July, and then dropped in September to about 1,500 ppg (table 3 and figure 2). However, there were small differences between the blocks. In block 7, the density remained almost constant throughout the growing season,

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[1] Personal communication with Kenelm Russell, Washington State Department of Natural Resources, Olympia, Wash., October 1982.

except for a slight decrease in September, while in block 8, Fusarium increased during June and July, then declined to a low of 756 ppg in September.

Initial seedling density was higher in fumigated than in unfumigated soils (28.6 versus 24.9 trees per square foot), and while density decreased gradually but steadily throughout the growing season in both, the rate of decrease was much less in fumigated soils (table 5 and figure 4). Although Fusarium was not isolated from dead or dying seedlings, the levels of Fusarium found are similar to those which have caused significant seedling mortality in other nurseries;[1] it is probable, then, that Fusarium, either alone or in combination with other agents, contributed to the mortality. At any rate, the difference in seedling density between fumigated and unfumigated soils, 20 percent in block 7 and 57 percent in block 8, for an overall difference of 36 percent, must be considered significant. In order to obtain an average seedling density of 27 trees per square foot without fumigation, 36 percent more seed would have to be planted. Comparing the cost of extra seed, $18,000, to the cost of fumigation, about $8,700, the relative cost of extra seeding is more than twice that of fumigation, even if no other benefits are considered.

Other benefits were visible but not measured; fewer weeds were found early in the season in fumigated soils, and by the end of the season, seedlings in fumigated soils were taller and greener than those in unfumigated soils--results similar to those reported by Boyd.[2] Boyd admitted that the reasons for improved seedling growth were unclear, but felt that reduced populations of pathogens may have resulted in better nutrition of seedlings, or in a reduction in sublethal infections which would stunt seedlings without killing them.

Other methods of reducing populations were not examined in this evaluation. Applications of captan and benomyl, at approximately twice the cost of fumigation, have been used in other nurseries to kill Fusarium without harming mycorrhizal fungi. Although mycorrhizae have been observed on 2-0 seedlings at ATN (4500 letter, July 30, 1979, from Jerry Riffle to Ed Wood), mean density is considered very low, possibly because of high soil pH. In addition, the fact that the nearest forested area is several miles to leeward of prevailing winds would probably severely restrict the amount of natural inoculum available.

CONCLUSIONS AND RECOMMENDATIONS

We conclude that the benefits gained from fumigation of blocks 7 and 8, namely higher seedling survival rate and quality, more than justified the expense of fumigation. We feel that seedlings should be

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[1] Ibid.

[2] Boyd, R. J. 1971. Effects of soil fumigation on production of conifer nursery stock at two northern Rocky Mountain Nurseries. USDA Forest Service Res. Pap. INT-91. 19 pp.

examined at the end of the second growing season in order to compare final seedling densities and quality. We recommend that block 5, to be seeded in 1983, be fumigated in the spring in 1983 using the same materials and techniques as were used in 1982.

TABLE 1.--Means ($\bar{x}$), standard errors ($s\bar{x}$), and confidence limits (c.l.) of population estimates for Fusarium sp. before and after fumigation at ATN

	Block	$\bar{x}$	$s\bar{x}$	c.l. (p=0.01)
Prefumigation	7	2,698.0 ab[1]	420.5	± 1227.8
	8	1,584.3 cd	182.2	± 531.9
Postfumigation	7	149.0 a	53.5	± 156.2
	8	47.1 c	19.6	± 57.3
End of season	7	509.8 b	249.8	± 729.3
	8	196.1 d	73.3	± 214.1

[1]Sample means followed by the same letter in a single column are significantly different, p=0.01, according to the t test.

TABLE 2.--Means ($\bar{x}$) and standard errors ($s\bar{x}$) of population estimates of Pythium sp. before and after fumigation at ATN

	Block	$\bar{x}$	$s\bar{x}$
Prefumigation	7	1.79	.64
	8	2.52	.81
Postfumigation	7	1.35	.78
	8	1.37	.53
End of season	7	.58	.33
	8	.04	.04

TABLE 3--Means ($\bar{x}$) and standard errors ($s\bar{x}$) of monthly population of *Fusarium* sp. in fumigated and unfumigated soil at ATN

		Block 7		Block 8		Combined	
		$\bar{x}$	$s\bar{x}$	$\bar{x}$	$s\bar{x}$	$\bar{x}$	$s\bar{x}$
May -	unfumigated	2,577.8	379.7	1,400.0	313.2	1,988.9	294.3
	fumigated	22.2	22.1	44.4	28.1	33.3	17.4
June -	unfumigated	2,600.0	422.7	2,111.1	388.6	2,355.6	283.5
	fumigated	44.5	44.5	177.8	74.4	111.1	45.9
July -	unfumigated	2,400.0	424.4	2,155.6	406.5	2,277.8	282.6
	fumigated	88.9	88.9	155.6	80.1	122.2	57.9
August -	unfumigated	2,644.4	312.5	1,600.0	262.2	2,122.2	250.2
	fumigated	466.7	187.8	133.3	48.7	300.0	105.3
September -	unfumigated	2,200.0	398.1	755.6	444.4	1,477.8	289.6
	fumigated	200.0	75.0	66.7	45.5	133.3	46.4

TABLE 4--Means ($\bar{x}$) and standard errors ($s\bar{x}$) of monthly populations of *Pythium* sp. in fumigated and unfumigated soil at ATN

		Block 7		Block 8		Combined	
		$\bar{x}$	$s\bar{x}$	$\bar{x}$	$s\bar{x}$	$\bar{x}$	$s\bar{x}$
May -	unfumigated	0.87	0.87	0.36	0.25	0.62	0.44
	fumigated	1.23	0.97	3.01	1.22	2.12	0.79
June -	unfumigated	0.25	0.16	0.60	0.34	0.42	0.18
	fumigated	0	0	0.12	0.12	0.06	0.06
July -	unfumigated	0.25	0.16	0	0	0.13	0.08
	fumigated	0	0	0	0	0	0
August -	unfumigated	0.86	0.58	1.55	0.39	1.21	0.35
	fumigated	1.11	0.83	0.36	0.36	0.74	0.44
September -	unfumigated	0.25	0.16	0.36	0.24	0.31	0.14
	fumigated	0.38	0.38	0.12	0.12	0.25	0.19

TABLE 5--Means ($\bar{x}$) and standard errors ($s_{\bar{x}}$) of monthly tree densities (trees per square foot) fumigated and unfumigated soil at ATN

	Unfumigated		Fumigated	
	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$
Block 7				
June	25.85	3.45	27.41	3.04
July	24.67	3.29	27.89	3.15
August	21.62	2.65	25.73	3.46
September	21.10	2.48	25.31	3.16
Block 8				
June	23.87	1.78	29.08	1.58
July	22.72	2.02	29.33	1.15
August	19.94	1.45	29.16	1.62
September	18.96	1.55	29.24	1.49
Blocks 7 and 8 combined				
June	24.86	1.88	28.60	1.67
July	23.70	1.86	28.61	1.61
August	20.76	1.47	27.44	1.89
September	20.03	1.43	27.28	1.77

FIGURE 1

PLOT LOCATION MAP

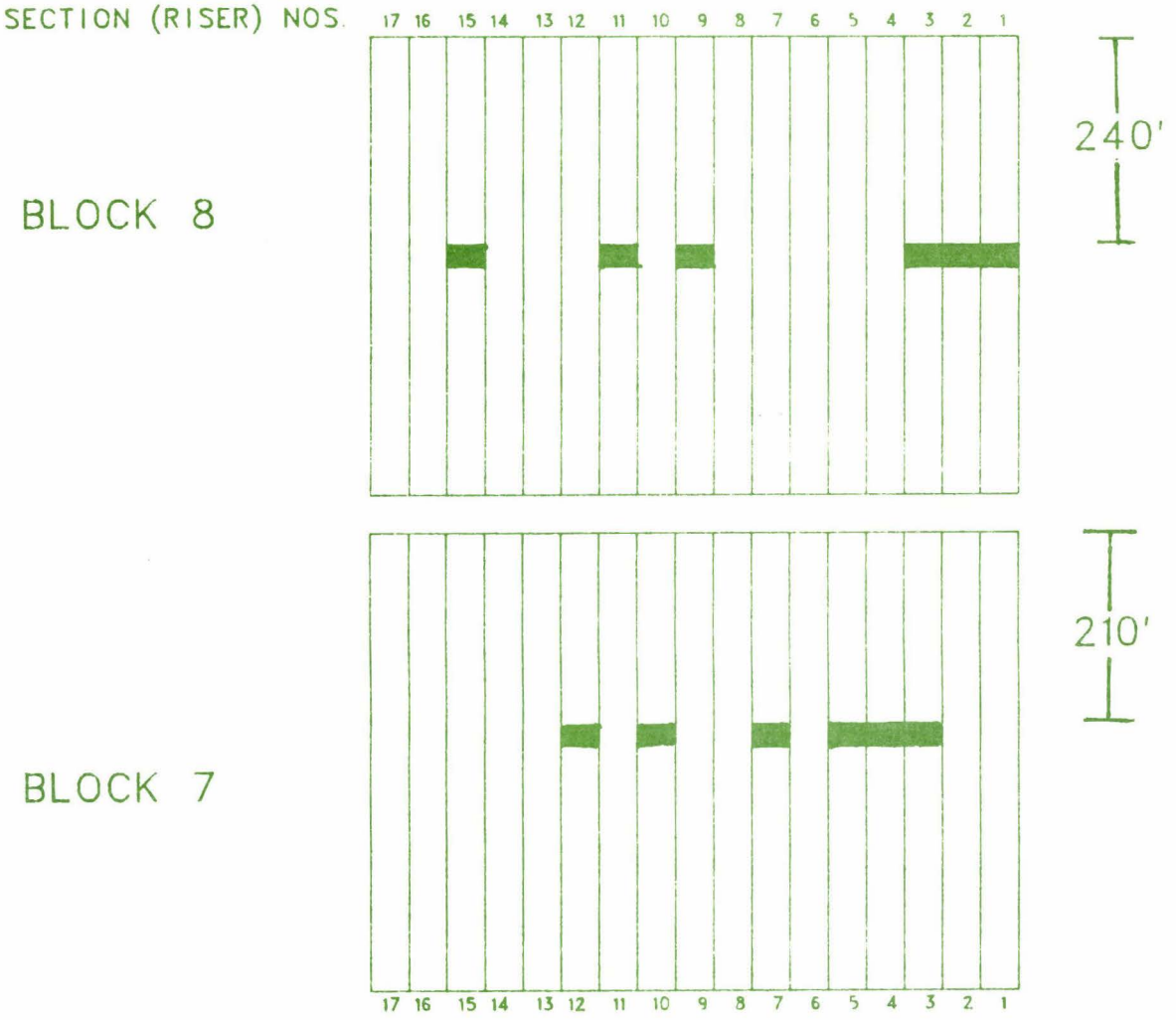


FIGURE 2.--MEAN MONTHLY POPULATIONS OF FUSARIUM SPP. AT THE ALBUQUERQUE TREE NURSERY

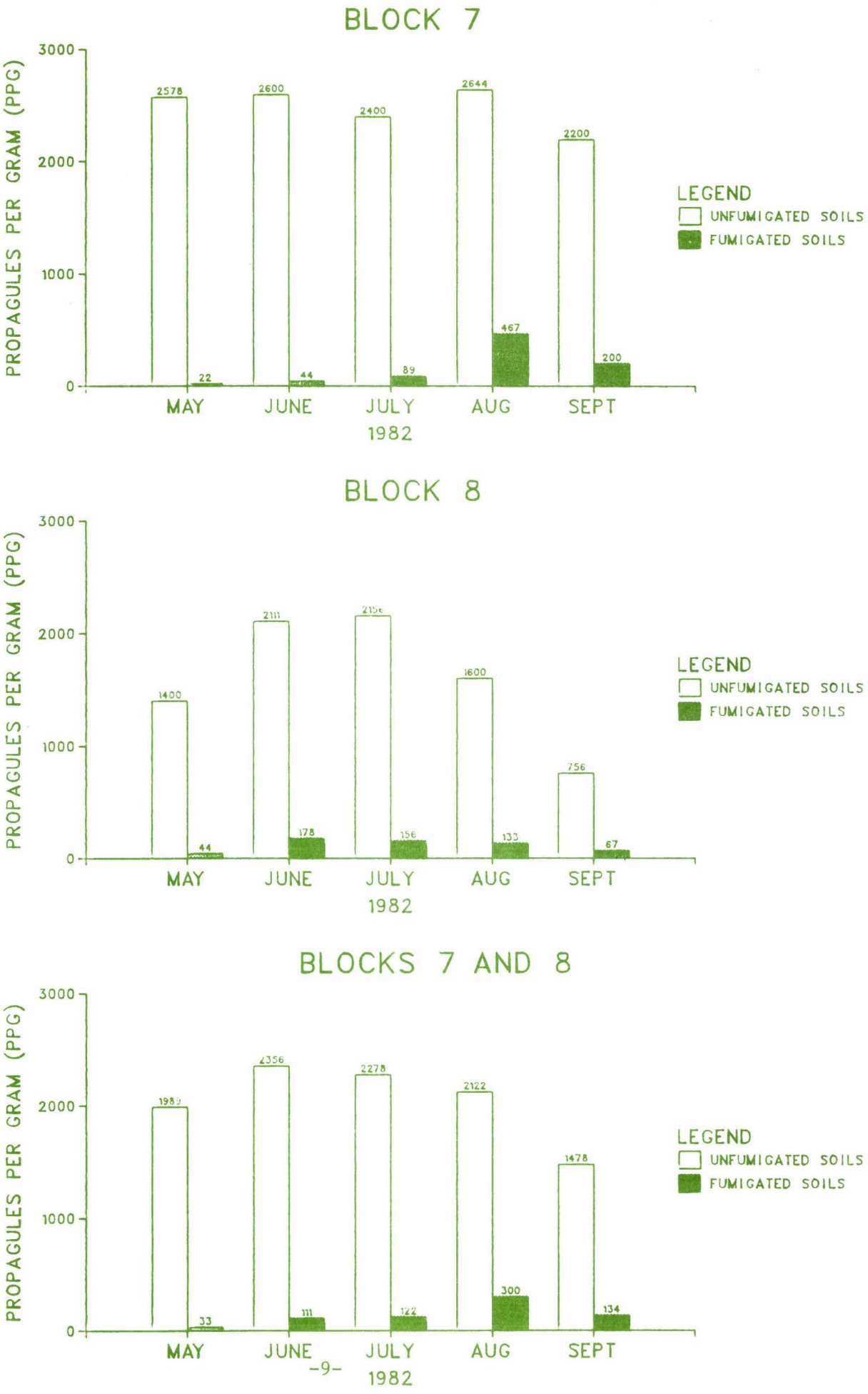
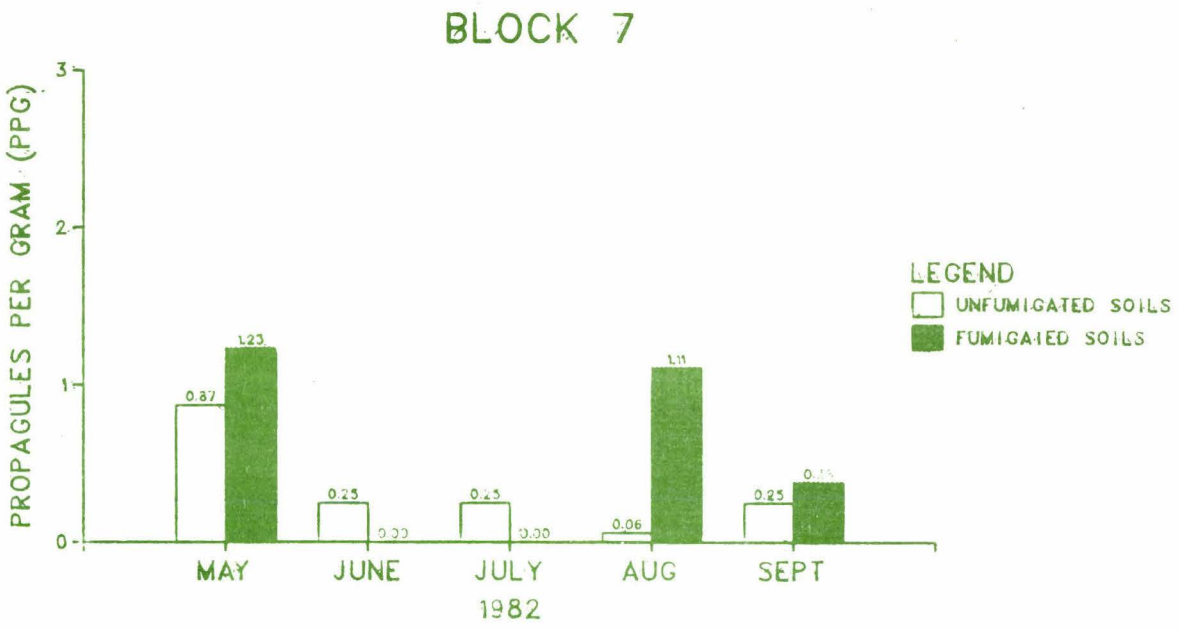
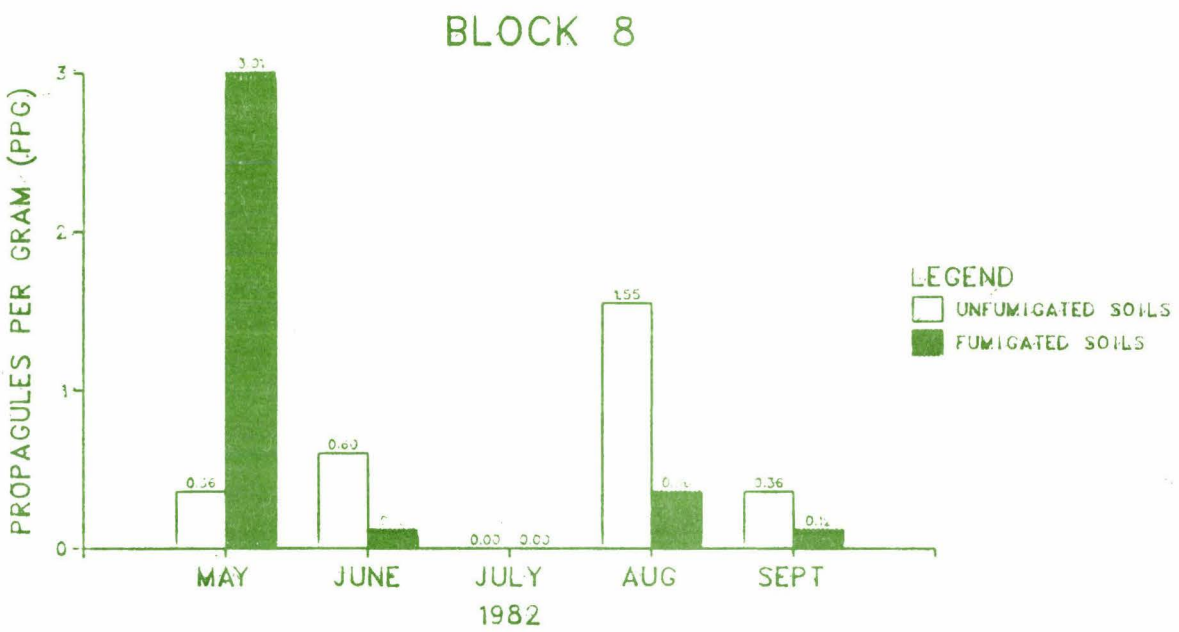


FIGURE 3.--MEAN MONTHLY POPULATIONS OF PYTHIUM SPP. AT THE ALBUQUERQUE TREE NURSERY





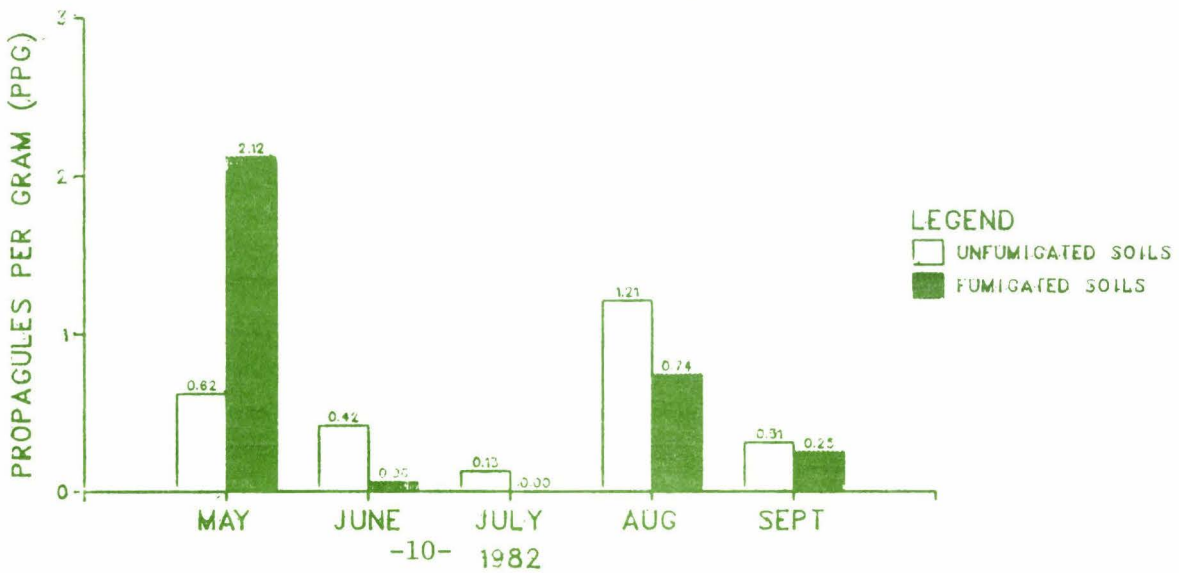
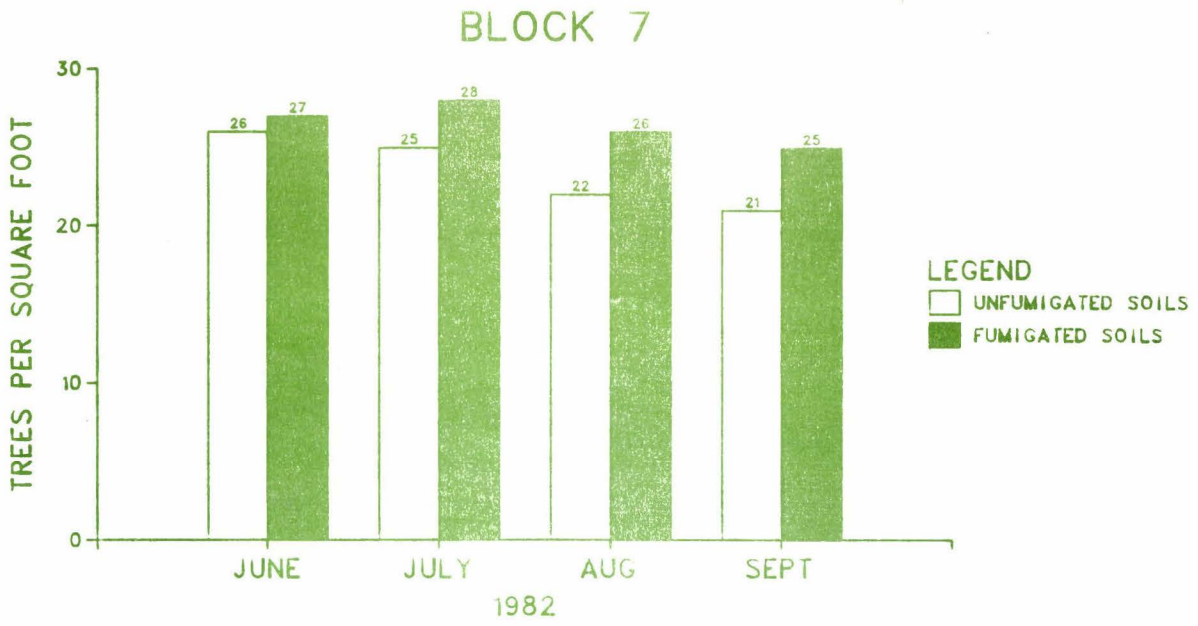
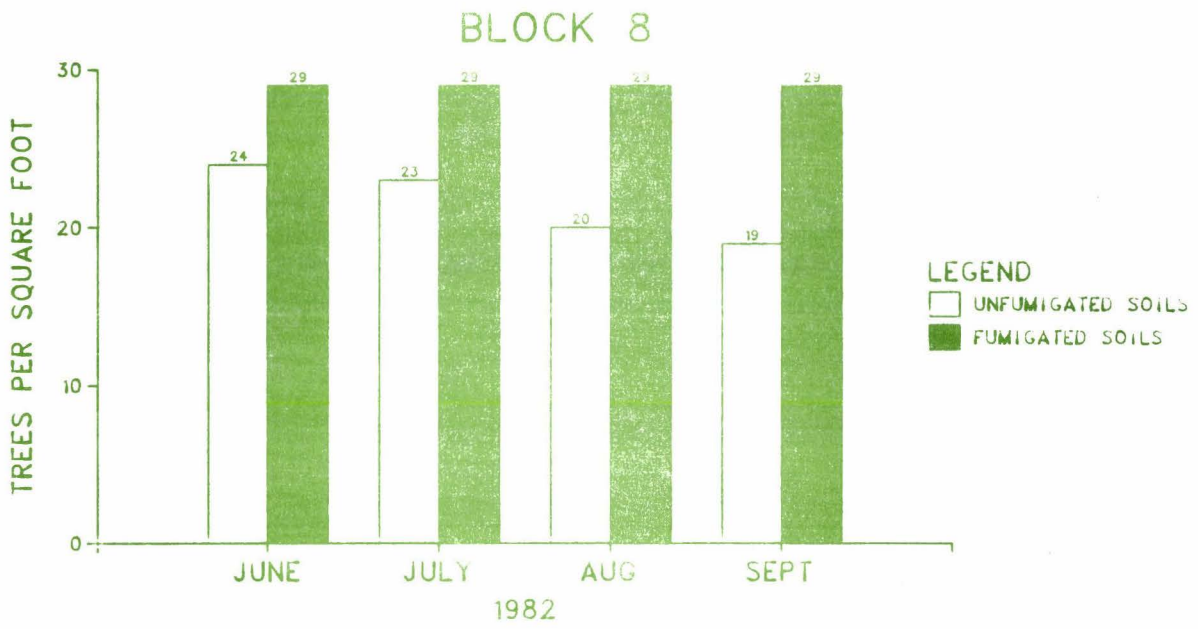
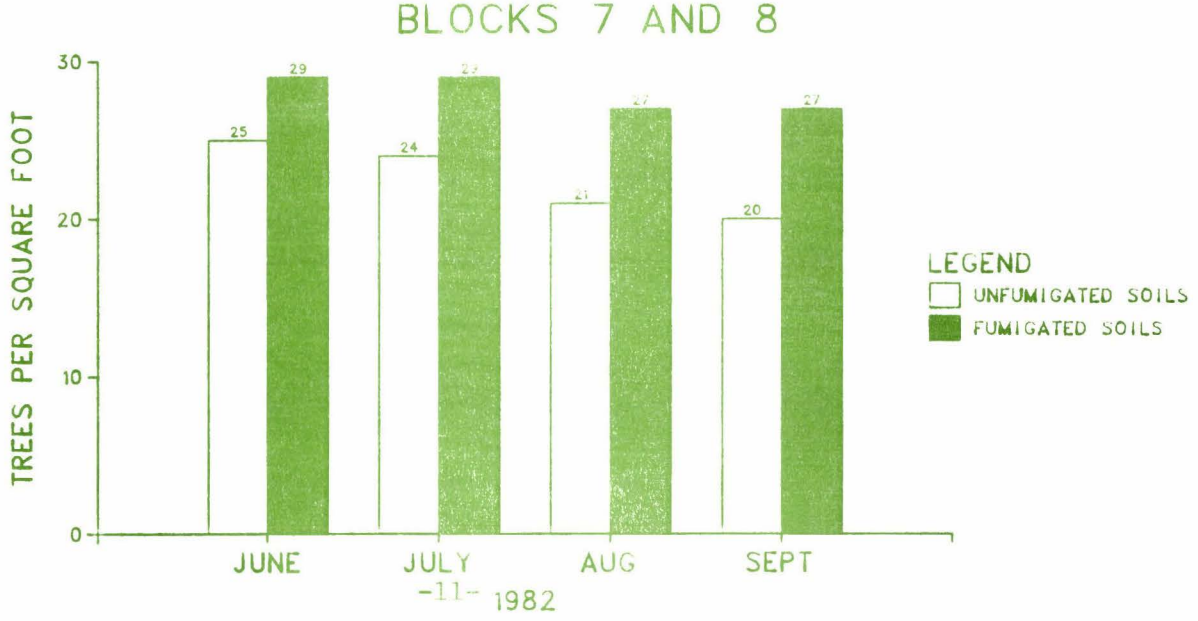


FIGURE 4.--MEAN MONTHLY TREE DENSITIES AT THE ALBUQUERQUE TREE NURSERY







APPENDIX I

NURSERY SOIL-BORNE PATHOGEN SURVEY PROCEDURES

I. SOIL COLLECTION

Soil was collected by walking down the center of the sections to be sampled and inserting a soil core extractor 4 to 6 inches vertically into the soil. The soil core was placed into a collection container. Soil cores were taken every 5 to 10 paces to the end of the sampled section. The soil cores in the collection container were broken up and poured into a Ziploc bag that was labeled with block and section numbers, date collected, and the treatment, either fumigated or unfumigated and pre- or post-fumigation. The labeled bags were stored in a cooler with blue ice until treated further.

II. PROCEDURE FOR DETERMINATION OF PYTHIUM SPP. IN SOIL

A. The selective medium recipe for *Pythium* spp. is as follows:

Distilled water	782 ml
Agar (must be granulated)	32 g
Sucrose	24 g
$NaNO_3$	1.6 g
KH_2PO_4	0.8 g
$MgSO_4:7H_2O$	0.4 g
KCl	0.4 g
Yeast extract	0.4 g
$FeSO_4$	0.8 ml of 1 g in 100 ml water (.008 g)
Rose Bengal	8 ml of 0.3 g in 100 ml (.024 g)
Autoclave, then add:	
PCNB (Terraclor 75% powder)	8 ml of 1.3 g in 100 ml (.104 g)
Streptomycin sulfate	0.4 ml of 5 g in 100 ml (5%)
50% Lactic acid	2 drops from 5 ml pipette

Mycostatin (Nystatin)	0.8 ml of 500,000 units dissolved in 5 ml water (enough for 6 bottles)

B. Preparation of Soil for Pythium spp. Determination

For each sample, soil was passed through a large screen (14 mesh/inch) into a labeled paper cup until the cup was one-half to three-fourths full. A clean, dry screen was used for each sample. Five grams of this soil was measured into a labeled 250 ml Erlenmeyer flask to be used for Pythium inoculation. An additional 5 grams of each sample was ovendried to determine the actual dry weight of soil used for each sample (for calculation of propagules per gram of soil). The remaining soil was set aside to air-dry for at least 5 days.

C. Pythium Inoculation

To each flask of soil, 10 ml of sterile 0.5-percent water agar was added. The water agar was kept at about 55° C in a hot water bath because it sets up rapidly. For this reason, only one flask was treated at a time. The flask of soil agar was swirled to mix contents thoroughly, and 1 ml was pipetted onto each of three labeled plates for each sample. A widemouthed delivering pipette was used to minimize clogging due to soil particles. Three ml were pipetted, and then approximately 1 ml was dispensed onto each of the three plates. If more than 1 ml was dispensed onto a plate, the excess was poured onto the next plate without affecting calculations, since the populations in all three plates were totaled. Three plates were used in order to reduce colony density and facilitate counting. The soil agar was spread evenly over the plate.

D. Pythium Incubation (3 days)

Incubation for Pythium required 3 days at room temperature in the dark.

E. Reading Pythium

Pythium spp. typically are featherlike, grow into the selective medium, and become dark pink as the hyphae take up the Rose Bengal. The aerial hyphae, water agar, and soil were scraped gently from the face of the medium with a finger or square-edged utensil (spoon) under running water. Care was taken to avoid gouging the medium. Cleaned plates were inverted on a light box (or over a fluorescent light source) and the pink feathery colonies were circled with a grease pencil and counted.

F. Calculating PPG of Pythium in Soil

$$\frac{\text{3 ml agar}}{\text{sample}} \times \frac{\text{5 g soil}}{\text{10 ml agar}}^{*} = \text{sample/1.5 g soil}$$

Total colonies (number of propagules per sample) ÷ 1.5 grams of soil per sample = propagules per gram of soil.

* Actual dry weight of soil as determined by ovendrying another 5 grams for each soil sample.

III. Procedure for Determination of Fusarium spp. in Soil

A. The selective medium recipe for Fusarium spp. consists of:

Distilled water	775 ml
Agar (may be flaked)	16 g
Peptone	12 g
KH_2PO_4	0.8 g
$MgSO_4:7H_2O$	10 ml of 0.4 g in 100 ml

The above ingredients are autoclaved. Then the following are added:

PCNB (Terraclor 75%)	0.6 g (15 ml of 4 g in 100 ml)
Neomycin sulfate	8 ml of 1 gm in 100 ml (1%)
Streptomycin sulfate	8 ml of 5 g in 100 ml (5%)

Plates must dry at least 4 days before bagging or using. Selectivity is retained for a few months if stored in plastic bags in the refrigerator.

B. Fusarium spp. Inoculation

After soil was air-dried in labeled paper cups for at least 5 days, 0.1 gram of soil was passed through a small screen (30 mesh/inch) and put in a labeled 250 ml Erlenmeyer flask for each sample. A clean, dry screen for each sample was used. Forty ml of sterile 0.1-percent water agar was poured into each flask. The flask was swirled and set aside for 30 minutes. The 0.1-percent water agar was too dilute and did not set up. For each sample, 1 ml of soil agar was dispensed onto each of three labeled plates using the same technique as described under Pythium spp. inoculation. The entire surface of the medium was covered by tipping and shaking the plate. The pipette was sterilized by rinsing with ethanol.

C. Fusarium spp. Incubation (5 days)

Inoculated plates were left for 5 days at room temperature under a bank of fluorescent lights about 2 feet above the plates. Light was required 24 hours per day.

D. Reading Fusarium

Fusarium spp. colonies are variously colored and textured. Microscopic identification is required until familiarization with colony appearance is acquired. Fusarium colonies at the Albuquerque Tree Nursery are pink and radiate out in a circular shape from the point of origin.

E. Calculating PPG of Fusarium in Soil

$$\frac{3 \text{ ml agar}}{\text{sample}} \times \frac{0.1 \text{ g soil (air-dried)}}{40 \text{ ml agar}} = \text{sample}/0.0075 \text{ g soil}$$

Total colonies (number of propagules per sample) ÷ 0.0075 = propagules per gram of soil.